THE EPIDEMIOLOGY OF A RABBIT RESPIRATORY INFECTION.

IV. Susceptibility of Rabbits to Spontaneous Snuffles.

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In previous communications we have pointed out that cultures made from the nasal passages of all rabbits affected with snuffles and of about 60 per cent of the so called normal stock rabbits at The Rockefeller Institute show *Bacterium lepisepticum*.[1,2] These findings led us to look upon this microorganism as the probable incitant of snuffles and to attempt to establish relationship between the presence of the bacterium in the nasal passages and the onset of the clinical symptoms. We had in mind also to determine whether accessory influences affect the susceptibility of rabbits to this disease.

Dr. Brown, Dr. Pearce, and Dr. Van Allen placed at our disposal 80 rabbits which were being used for experimental purposes.[3,4] The series contained normal, operated, and inoculated animals. Our method was to make cultures at frequent intervals of the nasal secretions of these rabbits in order to be able to compare the bacteriological findings present before spontaneous snuffles appeared with those as it developed. The swab technique employed has already been described.[1,2]

Spontaneous Clinical Snuffles.

Group 1. Normal Rabbits.—The nasal flora of twenty-two so called normal or control rabbits was determined at short intervals

[1] Webster, L. T., *J. Exp. Med.*, 1924, xxxix, 843.

[2] Webster, L. T., *J. Exp. Med.*, 1924, xxxix, 857.

[3] Brown, W. H., and Pearce, L., *Proc. Soc. Exp. Biol. and Med.*, 1922–23, **xx**, 476.

[4] Pearce, L., and Van Allen, C. M., *Tr. Assn. Am. Phys.*, 1923, **xxxviii**, 315.

for periods of 1 to 4 months. During this time, four of these animals developed snuffles. Table I shows the bacteriological findings in the four affected rabbits.[5]

In each instance, *Bacterium lepisepticum* was present preceding as well as during the attack of snuffles. In one instance (Rabbit 23) the organism predominated in cultures at least for 1 month before

TABLE I.

Spontaneous Snuffles in Normal Rabbits.

Rabbit 23.				Rabbit 139.			
Date.	Snuffles.	*Bacterium lepi-septicum.*	*B. bronchi-septicus.*	Date.	Snuffles.	*Bacterium lepi-septicum.*	*B. bronchi-septicus.*
Sept. 19	0	∞	0	Oct. 3	0	0	∞
" 26	0	∞	0	" 5	+	+	++
Oct. 17	++	∞	0	" 8	++	++	++
				" 11	++	++	++
				Nov. 8	++	0*	++*
				Autopsy.			
Rabbit 144.				Rabbit 176.			
Oct. 3	0	0	++	Oct. 17	0	0	∞
" 5	0	0	++	Nov. 8	0	0	∞
" 8	0	++	++	Dec. 12	0	0	0
" 11	+	∞	0	Jan. 29	+	∞	0
Nov. 8	++	∞	0	Mar. 8	+	∞	0
Autopsy.	++						

In thetables under Snuffles 0 indicates no snuffles; ±, questionable snuffles; +, moderate snuffles; ++, severe snuffles. Under *Bacterium lepisepticum* and *B. bronchisepticus* 0 indicates no colonies; +, 1 to 10 colonies; ++, 10 to 100 colonies; ∞ more than 100 colonies.

* Old plates inhibited. Growth of *Bacterium lepisepticum*. See Webster.[1]

the onset of symptoms. In another instance (Rabbit 139) the appearance of the bacterium coincided with the onset of symptoms

[5] Observations on the other animals of this series have been described already.[2] It was noted at that time that a certain number of them showed symptoms of chronic snuffles throughout the period of observation while others were clinically normal; that all those affected with snuffles carried *Bacterium lepisepticum* as the predominating organism in their nasal passages; and that 60 per cent of the normal rabbits carried *Bacterium lepisepticum* at one time or another.

and increased in number with the increase ot the symptoms. In still another animal (Rabbit 144) the organism, previously absent, was found in large numbers a few days preceding the symptoms, while in Rabbit 176 the appearance of large numbers of the bacterium and the onset of the snuffles coincided.

Group 2. Operated, Uninoculated Rabbits.—This group contains five rabbits, thyroidectomized or thymectomized during the 3 months period of observation. Following operation, two of these animals developed snuffles. Here again the coincidence of the appearance of *Bacterium lepisepticum* and of the symptoms was striking. In one instance, the bacterium was cultured in large numbers from the left naris just as a left-sided discharge appeared. The right side at this time was free both from snuffles and *Bacterium lepisepticum.* There had previously been three negative cultures covering a period of 1 month. The other rabbit suddenly showed the bacterium in the nares and developed snuffles after five negative cultures over a 5 weeks period.

Group 3. Inoculated Rabbits.—The nineteen rabbits in this group were inoculated with *Treponema pallidum* or with a malignant rabbit tumor during the 3 to 5 months period of observation. Eight of the animals developed snuffles within this time.

In every instance *Bacterium lepisepticum* was present in the nasal passages either before or after the inoculation and always before the onset of the snuffles. Rabbits 44 and 130 yielded positive cultures 2 months and Rabbits 10 and 29 about 3 weeks before symptoms appeared, while Rabbits 36 and 122 showed the organism and clinical symptoms practically concurrently.

Group 4. Operated and Inoculated Rabbits.—Thirty-four rabbits which had been thyroidectomized or thymectomized and then inoculated with either *Treponema pallidum* or with a malignant rabbit tumor were observed for 5 months during which time thirteen developed spontaneous snuffles (Table II). Rabbits 22, 125, 32, 30, and 43 showed *Bacterium lepisepticum* for 1 to 4 months prior to the onset of symptoms and Rabbits 19, 130, and 179 less than 1 week before them. On the other hand, Rabbits 41, 126, 131, and 137 presented questionable symptoms and occasional positive cultures for varying periods of time, following which severe snuffles developed

TABLE II.

Spontaneous Snuffles in Operated and Inoculated Rabbits.

Rabbit 19.				Rabbit 22.				Rabbit 30.				Rabbit 32.			
Date.	Snuffles.	*Bacterium lepisepticum.*	*B. bronchisepticus.*	Date.	Snuffles.	*Bacterium lepisepticum.*	*B. bronchisepticus.*	Date.	Snuffles.	*Bacterium lepisepticum.*	*B. bronchisepticus.*	Date.	Snuffles.	*Bacterium lepisepticum.*	*B. bronchisepticus.*
Sept. 19	0	∞	0	Sept. 19	0	++	0	Sept. 20	0	++	0	Sept. 20	0	∞	0
" 26	++	∞	0	" 26	0	++	+	" 26	0	++	0	" 26	0	+	++
Oct. 17	++	∞	0	Oct. 17	0	0	++	Oct. 17	0	∞	0	Oct. 17	0	0	++
Nov. 2	Operation.			" 29	Operation.			" 26	Operation.			" 26	Operation.		
" 9	++	∞	0	Nov. 12	0	0	++	Nov. 12	++	∞	0	Nov. 8	++	∞	0
" 15	Inoculation.			" 15	Inoculation.			" 15	Inoculation.			" 15	Inoculation.		
Dec. 17	0	0	∞	Dec. 20	0	++	0	Dec. 14	±	++	++	Dec. 20	++	∞	0
Jan. 20	0	0	++	Jan. 15	++	∞	0	Jan. 15	0	∞	0	Jan. 15	++	∞	0
Autopsy.				Autopsy.				Autopsy.				Autopsy.			
Rabbit 41.				Rabbit 43.				Rabbit 125.				Rabbit 126.			
Sept. 20	0	0	0	Sept. 20	0	0	∞	Oct. 3	0	0	0	Oct. 3	0	+	0
" 26	±	+	0	" 26	0	0	∞	" 5	0	0	0	" 5	±	0	0
Oct. 19	±	0	0	Oct. 19	0	++	++	" 8	0	∞	0	" 8	±	++	0
Nov. 2	Operation.			" 26	Operation.			" 11	±	+	0	" 11	±	0	0
" 9	+	∞	0	Nov. 9	++	∞	0	" 16	Operation.			" 16	Operation.		
" 15	Inoculation.			" 15	Inoculation.			" 22	0	0	0	" 22	0	++	0
Dec. 17	++	∞	0	Dec. 17	++	∞	0	" 29	Inoculation.			" 29	Inoculation.		
Jan. 20	++	∞	0	" 26	++	∞	0	Nov. 17	0	∞	0	Nov. 8	+	∞	0
Autopsy.				Autopsy.				Dec. 14	0	+	0	Dec. 14	±	∞	0
								Jan. 28	++	∞	0	Jan. 28	++	∞	0
								Mar. 8	++	∞	0	Mar. 8	+	∞	0

Rabbit 130.				Rabbit 131.				Rabbit 137.				Rabbit 138.				Rabbit 179.			
Date.	Snuffles.	*Bacterium lepisepticum.*	*B. bronchisepticus.*	Date.	Snuffles.	*Bacterium lepisepticum.*	*B. bronchisepticus.*	Date.	Snuffles.	*Bacterium lepisepticum.*	*B. bronchisepticus.*	Date.	Snuffles.	*Bacterium lepisepticum.*	*B. bronchisepticus.*	Date.	Snuffles.	*Bacterium lepisepticum.*	*B. bronchisepticus.*
Oct. 3	0	0	++	Oct. 3	±	∞	0	Oct. 3	0	0	0	Oct. 3	0	∞	0	Oct. 17	0	0	∞
" 5	0	∞	0	" 5	±	0	++	" 5	0	++	0	" 5	0	∞	++		Operation.		
	Operation.			" 8	0	0	++	" 8	0	0	0	" 8	0	0	∞	" 22	0	0	++
" 8	++	∞	+	" 15	Operation.			" 11	0	0	0	" 11	0	0	++	" 27	Inoculation.		
" 11	++	∞	0	" 22	0	0	∞	" 16	Operation.			" 16	Operation.			Nov. 7	0	0	++
" 22	++	∞	0	" 29	Inoculation.			" 22	0	0	0	" 22	0	0	++	Dec. 12	+	++	++
" 29	Inoculation.			Nov. 8	++	∞	0	" 29	Inoculation.			" 29	Inoculation.			Jan. 28	++	∞	0
Nov. 8	++	∞	0	Dec. 11	++	∞	0	Nov. 7	0	0	0	Nov. 7	0	0	++	Mar. 8	++	∞	0
Dec. 14	++	∞	0	Jan. 29	++	++	0	Dec. 12	++	∞	0	Dec. 12	L.						
Jan. 29	++	∞	0	Mar. 8	++	∞	0	Jan. 28	++	∞	0		++	∞	0				
Mar. 8	++	∞	0					Mar. 8	+	∞	0		R.						
													0	0	0				
												Jan. 28	++	∞	0				
												Mar. 8	++	∞	0				

and the organism was recovered repeatedly in almost pure culture. Rabbit 138 presented a striking example (see Group 2, Rabbit 162) of early unilateral nasal discharge associated with *Bacterium lepisepticum* on the affected side only, followed later by an abundant discharge and large numbers of microorganisms on both sides.

In brief, then, throughout this entire series, *Bacterium lepisepticum* was present at some time in the nasal passages prior to the onset of snuffles and continued as the predominating organism throughout the clinical course of the disease.[6] A comparison of the percentage incidence of snuffles among the 22 control rabbits of Group 1 and the 58 operated or inoculated rabbits of other groups gives a 20 per cent incidence and one of 40 per cent respectively, thus indicating that the experimental procedures tended to impair resistance to this respiratory infection.

Disappearance of Clinical Snuffles.

Since it appears that the onset of spontaneous snuffles in rabbits is preceded by a longer or shorter period in which *Bacterium lepisepticum* is present in the nasal passages, and that this organism persists throughout the course of the disease, we undertook to ascertain by cultures whether, on spontaneous recovery, the bacterium could still be found. We selected from the above series of 80 rabbits those which during the 5 month period of observation had spontaneously recovered from snuffles. Table III summarizes the findings in six such instances.

Rabbit 19 developed severe snuffles 1 week or more after the appearance of *Bacterium lepisepticum;* 2 weeks later, the nasal discharge cleared up entirely and the organism was not again found. At autopsy not only were the nasal cultures negative but the mucous membranes seemed normal. In Rabbit 30, the bacterium was cultivated from the nares 6 weeks before the onset of snuffles. All symptoms disappeared 5 weeks later, but though at autopsy the nasal passages were normal, the organism was still present. Rabbit

[6] Data relating to the prevalence of *B. bronchisepticus* have been included in the tables because certain workers have considered this organism as the incitant of snuffles. Our studies provide no basis for this view but controvert it.

33 showed severe snuffles associated with *Bacterium lepisepticum* in nearly pure culture for at least 7 weeks. Both clinical symptoms and organisms then disappeared and at autopsy the nasal passages were normal and cultures were negative. Severe snuffles was associ-

TABLE III.

Recovered Cases.

Rabbit 19.			
Date.	Snuffles.	*Bacterium lepisepticum.*	*B. bronchisepticus.*
Sept. 19	0	∞	0
" 26	++	∞	0
Oct. 17	++	∞	0
Nov. 2	Operation.		
" 9	++	∞	0
" 15	Inoculation.		
Dec. 17	0	0	++
Jan. 20	0	0	++
Autopsy.			

Rabbit 30.			
Date.	Snuffles.	*Bacterium lepisepticum.*	*B. bronchisepticus.*
Sept. 20	±	++	0
" 26	0	++	0
Oct. 17	0	∞	0
" 26	Operation.		
Nov. 2	++	∞	0
" 15	Inoculation.		
Dec. 14	±	++	++
Jan. 15	0	++	0
Autopsy.			

Rabbit 33.			
Date.	Snuffles.	*Bacterium lepisepticum.*	*B. bronchisepticus.*
Sept. 20	++	∞	0
" 26	++	∞	0
Oct. 19	+	∞	0
" 26	Operation.		
Nov. 9	++	∞	++
" 15	Inoculation		
Dec. 17	0	0	+
Jan. 15	0	0	+
Autopsy.			

Rabbit 119.			
Oct. 3	+	∞	++
" 5	+	?	+
" 8	++	∞	0
" 15	Operation.		
" 22	++	∞	0
" 29	Inoculation.		
Nov. 7	0	0	++
Dec. 12	0	0	0
Jan. 28	0	0	+
Mar. 8	0	0	+

Rabbit 148.			
Oct. 3	0	0	0
" 8	+	∞	0
" 11	0	+	0
Nov. 8	0	0	0
" 14	0	0	0
Autopsy.			

Rabbit 177.			
Oct. 17	++	∞	0
	Operation.		
" 22	++	∞	0
" 29	Inoculation.		
Nov. 8	++	∞	0
Dec. 14	0	∞	0
Jan. 29	0	0	++
Mar. 8	0	0	++

ated with many organisms in Rabbits 119 and 117 during the first 3 weeks of observation, after which the symptoms cleared up and the bacterium could no longer be cultivated from the nasal passages. The presence of symptoms and organisms was limited to a few days in Rabbit 148.

In these six instances, then, the subsiding of symptoms or recovery from snuffles was associated with a diminution in number or a complete disappearance of the bacterium from the nasal passages.

DISCUSSION.

The data presented in this paper tend to support our previously expressed view that the presence in the nasal passages of *Bacterium lepisepticum* bears an etiological relationship to snuffles as it occurs at The Rockefeller Institute. The facts here brought out, taken together with observations previously published,[1,2] have led us to the conclusion that certain strains of the bacterium, widely distributed among the rabbits at The Rockefeller Institute, are of a grade of virulence too low to induce epidemic outbreaks of infection attended by septicemia and pleuropneumonia, but are yet sufficiently active to excite snuffles in a not inconsiderable percentage of the population.

According to the observations reported in this and preceding papers we may divide the rabbit population into three groups according to their resistance to strains of *Bacterium lepisepticum* that are of low virulence: (1) the resistant 20 per cent which do not offer a favorable medium for the growth of the bacterium; (2) the less resistant 40 per cent which carry the organism in their nasal passages without acquiring snuffles; and (3) the least resistant 40 per cent in which snuffles develops.

CONCLUSIONS.

The onset of spontaneous snuffles in rabbits at The Rockefeller Institute is preceded by the appearance in the nasal passages of *Bacterium lepisepticum.*

The active stages of snuffles infection are associated with the presence in these passages of large numbers of this bacterium.

Spontaneous recovery from snuffles is associated with a diminution in number or a disappearance of these bacteria from the nasal passages.

Various experimental procedures reduce the resistance of rabbits to spontaneous snuffles.